PHYSICOCHEMICAL STRUCTURE OF THE STARCH GRANULE

I. THE STARCH GRANULE

Starch and glycogen (the storage material in animals and bacteria) are both polymers of α-D-glucose, but starch differs from glycogen in that starch consists of a highly ordered and dense packing of glucan chains organized within large, insoluble granules. The starch granules are formed in the amyloplast (see the chapter, "The Site of Starch Synthesis in Nonphotosynthetic Plant Tissues; The Amyloplast"), specialized in the synthesis and long-term storage of starch, or in the chloroplast (see the chapter, "Starch Accumulation in Photosynthesis Cells"), where starch serves as a temporary store of energy and carbon.

Starch granules vary in size, shape, composition, and properties (Table I), and they are a semicrystalline material. Because the starch granule has a high degree of order, when viewed in polarized light it shows birefringence, the maltese cross of Fig. 1.

The shape and size of the granules depend on the source. For example, pollen starch granules are about 2 μm in diameter and those from canna starch have diameters of up to 175 μm. Although the microscopic appearance of starch granules (Fig. 2) is sufficiently characteristic to allow the identification of the botanical source of the polysaccharide, in each tissue there is a range of sizes and shapes. For example, in barley starch there are two populations of granules: one is composed of large lenticular granules with diameters between 15 and 35 μm, and another of small spherical granules with diameters between 1 and 10 μm. In general, the diameter of the starch granule changes during the development of the reserve tissue. In addition to size and shape, there are also some fine features that are characteristic of each species (e.g., the "growth rings" seen in potato starch), which help to identify the botanical source of the starch upon microscopic examination.

II. AMYLOSE AND AMYLOPECTIN

At least two polymers can be distinguished within the starch granule: amylose, which is essentially linear; and amylopectin, which is highly

TABLE I

COMPARISON OF STARCHES USED COMMERCIALLY[a]

Type of starch, composition and properties	Potato	Maize		Wheat	Tapioca
		Wild type	Waxy		
Starch granules					
Shape	Oval–spherical	Round–polygonal	Round–polygonal	Round–lenticular	Round–polygonal
Diameter, range (μm)	5–100	2–30	2–30	0.5–45	4–35
Composition					
Moisture[b]	19	13	13	13	13
Lipids[c]	0.1	0.8	0.2	0.9	0.1
Nitrogen compounds[c]	0.1	0.35	0.25	0.4	0.1
Ash[c]	0.35		0.1	0.2	0.1
Phosphorus[c]	0.08	0.02	0.01	0.06	0.01
Starch-bound phosphorus[c]	0.08		0	0	0
Pregelatinized starches					
Taste and odor substances	Low	High	Medium	High	Very low
Amylose					
Amylose content[c]	21	28	1	28	17
Degree of polymerization (DP)					
Number average DP	4900	930	—	1300	2600
Weight average	6400	2400	—	—	6700
Apparent DP distribution	840–22,000	400–15,000	—	250–13,000	580–22,000

Amylopectin					
Degree of polymerization (DP)					
DP × 10^4 (range)	0.3–3	0.3–3	0.3–3	0.3–3	0.3–3
Gelatinization					
Pasting temperature, C°	60–65	75–80	65–70	80–85	60–65
Swelling power at 95°C	1153	24	64	21	71
Solubility at 95°C	82	25	41	48	23
Starch pastes					
Paste viscosity	Very high	Medium	High	Low	High
Water binding[d]	24	15	22	13	20
Paste texture	Long	Short	Long	Short	Long
Paste clarity	Nearly clear	Opaque	Fairly clear	Cloudy	Quite clear
Resistance to shear	Low	Medium	Low	Medium	Low
Rate of retrogradation	Medium	High	Very low	Medium	Low
Main commercial uses	Food, paper adhesives	Sugar, paper, corrugated board	Food, adhesives	Sugar, bakery	Food, adhesives

[a] Data from Swinkels (1989).
[b] Moisture at 65% RH and 20°C.
[c] % of dry matter.
[d] Water-binding capacity in parts of water per part of dry native starch to reach similar hot viscosity after cooking.

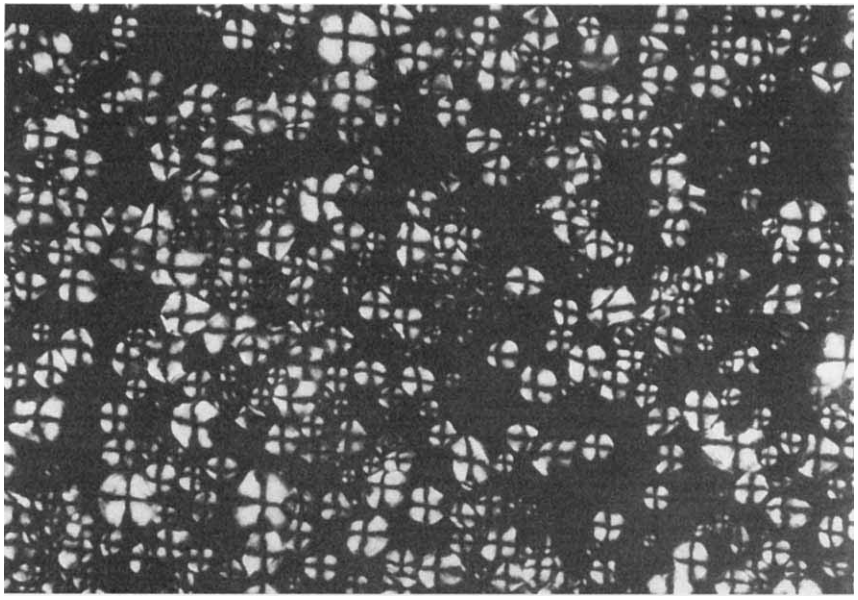

FIG. 1. The birefringence (maltese cross) shown by maize starch illuminated with polarized light, 700×. From Fitt and Snyder (1984).

branched (Fig. 3, Table II). Amylose is found mainly as linear chains of about 1500 units of α-D-glucopyranosyl residues linked by α-(1→4) bonds (molecular weight around 250,000; the molecular weight of an anhydroglucose residue is 162), but the number of anhydroglucose units varies widely with plant species and stage of development. Some molecules found in the amylose fraction are branched to a small extent (1→6 α-D-glucopyranose; 1 per 1000 or 1500 glucose residues). In contrast, amylopectin, which usually constitutes about 70% of the starch granule, is more highly branched, with about 4 to 5% of the glucosidic linkages being α-1→6 (Fig. 3). Methylation followed by acid hydrolysis shows that there is one nonreducing end group for every 20 to 25 D-glucose residues; this has been confirmed by the periodate oxidation method. These results are only compatible with a highly branched molecule and explain why amylopectin does not form threads or films in the same way as amylose. From the hydrolysis products, about 3% are 2,3-di-*O*-methyl-D-glucose, indicating that some glucose residues are joined to others through $C_{(6)}$ as well as through $C_{(1)}$ and $C_{(4)}$, and these units constitute the branch points. This is confirmed by the isolation of isomaltose and panose (α-D-G_p1–6α-D-G_p1-4-D-G_p) after partial hydrolysis of amylopectin. Thus, the average chain length of amylose is about 1500

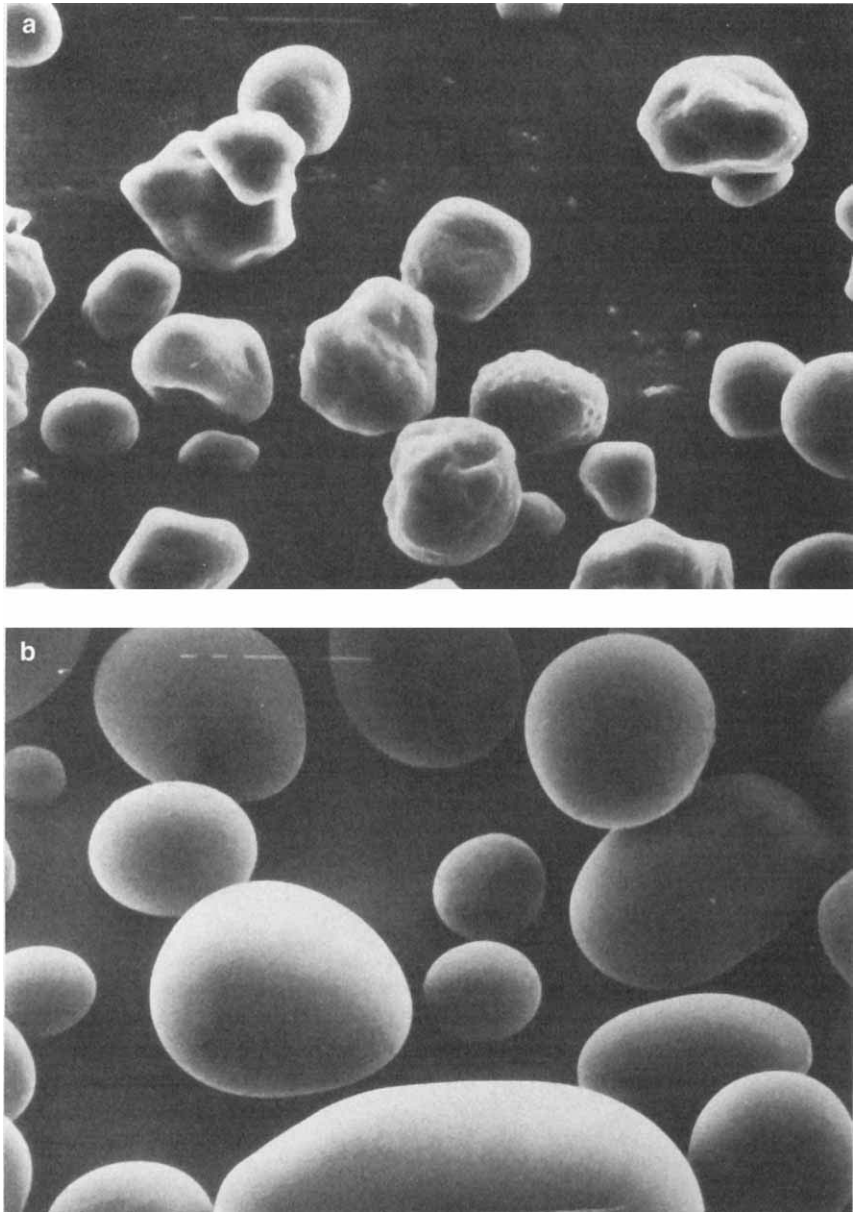

FIG. 2. Scanning electron micrographs of starch granules from (a) maize, 1500×; (b) potato, 1500×; (c) rice, 5000×; and (d) tapioca, 1500×. From Fitt and Snyder (1984).

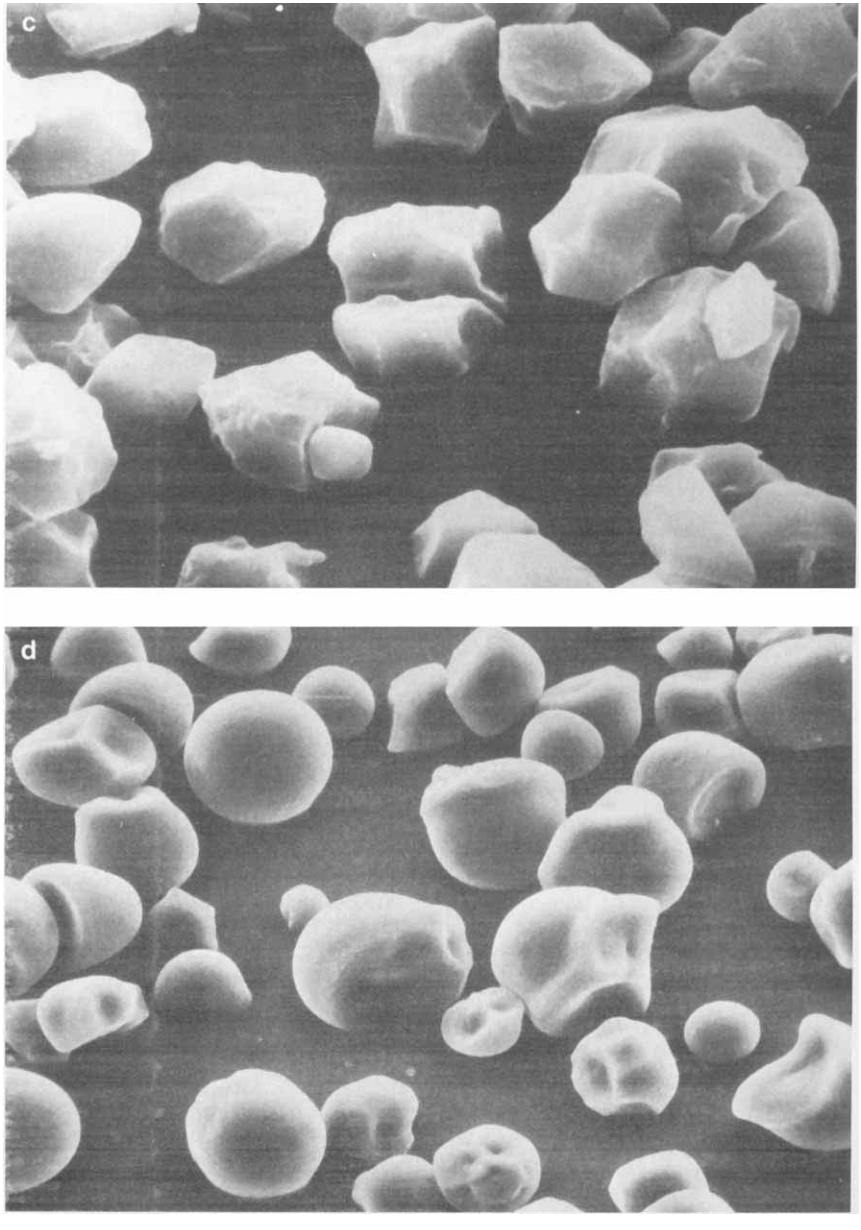

FIG. 2. (*Continued*).

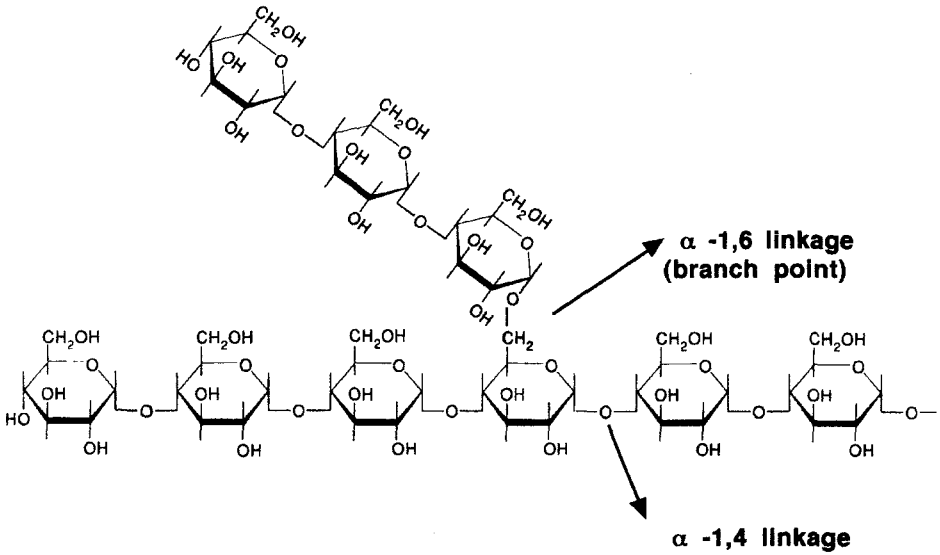

FIG. 3. The α-(1,4) and α-(1,6) glycosidic linkages between the glucosyl units present in starch.

TABLE II

PROPERTIES OF THE STARCH COMPONENTS AMYLOSE AND AMYLOPECTIN[a]

Property	Amylose			Amylopectin	Intermediate fraction
	Whole	Linear	Branched		
Branch linkage (%)	0.2–0.7	0	0.2–1.2	4.0–5.5	2–3.5
Average chain length (CL)	100–550	800	140–250	18–25	30–50
Average degree of polymerization (DP)	700–5000		10^3–10^4	10^4–10^5	10^2–10^4
λ_{max} (nm)	640–660			530–570	570–580
Blue value[b]	1.2–1.6			0–0.2	0.3–0.7
Iodine affinity (g per 100 mg)	19–20.5			0–1.2	2–10
Helix formation[c]	Yes	Yes	Yes	No	No
β-Amylolysis limit	70–95	100	40	55–60	57–75

[a] Data from Hizukuri (1995).
[b] Blue value: absorbance at 680 nm of the iodine complex in controlled conditions.
[c] With 1-butanol.

glucose residues and, for amylopectin, the average chain length is about 20 to 25 units. A typical molecular weight for amylopectin is around 10^8, with about 600,000 glucose residues.

It should be noted that the different structures of amylose and amylopectin confer distinctive properties to these polysaccharides (Table II). The linear nature of amylose is responsible for its ability to form complexes with fatty acids, low-molecular-weight alcohols, and iodine; these complexes are called clathrates or helical inclusion compounds. This property is the basis for the separation of amylose from amylopectin: when starch is solubilized with alkali or with dimethylsulfoxide, amylose can be precipitated by adding 1-butanol and amylopectin remains in solution.

When an aqueous starch solution is left to stand for some time, partial precipitation occurs. This is known as retrogradation and is due to the separation of the amylose fraction. The linear molecules align themselves parallel to each other and become held together by hydrogen bonds. The aggregates increase in size until they exceed colloidal dimensions and therefore precipitate. Because of this tendency, it is difficult to work with amylose, and to keep it in solution, it is often necessary to keep it at a high pH and at relatively high temperatures. Conversely, amylopectin does not generally form complexes and is stable in aqueous solutions. In some plant varieties, a minor third fraction, referred to as "anomalous amylopectin" or "intermediate fraction" (Table II), may also be present and can complicate fractionation. This fraction has fewer branch linkages than normal amylopectin; that is, it has greater average chain length (Hizukuri, 1995).

The early work of Katz and colleagues in the 1930s established that starch can give a number of distinct types of X-ray patterns, depending on the source of the starch and the treatment to which the granules were subjected. In intact starch granules, three dominant patterns, named A, B, and C, can be observed (Fig. 4). In the 1940s, French and his co-workers, using flow dichroism and X-ray examination of the amylose–iodine complex, showed that the amylose molecule is in the form of a helix, as had been proposed earlier by Hanes. French *et al.* suggested that there were six D-glucose units in each turn, with the iodine atoms lying along the axis of the helix. In 1972, Kainuma and French pointed out that models based on a sixfold helix could not satisfy the experimental values obtained by X-ray crystallography for B-amylose, and they postulated the presence of double helices. In solutions containing suitable "guest" molecules, segments of amylose would complex to form single left-handed V-type helices with a hydrophobic cavity of about 0.5 nm in diameter. In I_2/KI solution, the guest molecules are polyiodide ions (mostly I_3- or I_5-). The color and λ_{max} of the complexes vary with chain length and analytic conditions, and the iodine binding capacity is around 20 g/100 g amylose.

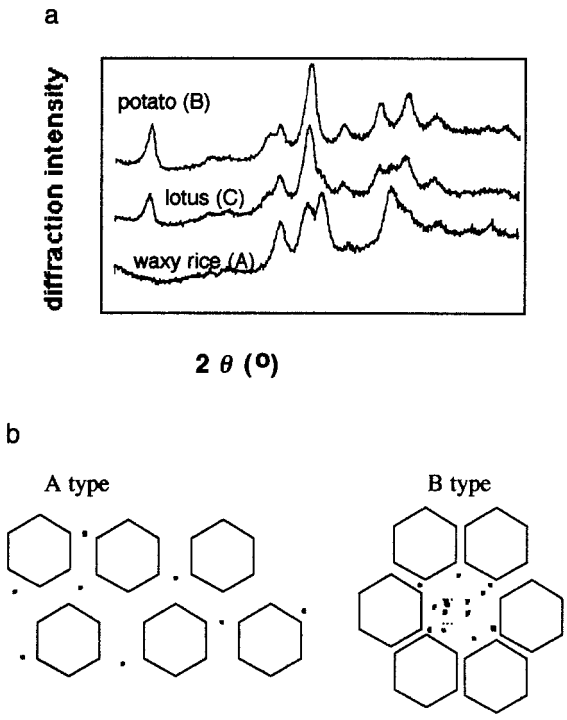

FIG. 4. (a) Diffractometer patterns of starch showing typical A, B, and C types of X-ray spectra. (b) Packing of double helices in the crystalline patterns proposed for the A and B types of starch. The C type would be a mixture (in varying amounts for different species) of A and B type of packing. After Hizukuri (1995).

The capacity of starch to stain blue-black with iodine suggests that some of the amylose is present in the starch in the V-form. The lipids present in cereal starch would bind to amylose if it were in the V-form, and yet X-ray analysis does not show the presence of the V-polymorph in cereal starches (i.e., most of the amylose would be in the amorphous form). The conclusion is that although a significant part of the amylose is probably in the helical form, the three-dimensional order necessary to give a crystalline diffraction pattern is absent. Indeed, the crystalline nature of starch is now attributed to the presence of amylopectin and not to amylose. Starch from waxy mutants contains only amylopectin (and no amylose), but this starch has the same degree of crystallinity and the same X-ray pattern as the regular starches that contain both components.

Starch granules are microcrystalline, comprising crystalline domains, non-crystalline domains, and possibly transitional regions. Native starch granules

have crystallinities estimated to range between 20 and 40%; this relatively low crystallinity is responsible for the low-quality X-ray diffractograms. Although it is generally thought that branching in a molecule is detrimental to crystallization, it seems that in the case of starch, amylopectin, which is the branched molecule, and not the almost linear amylose, is the fraction responsible for the crystalline nature of starch. Indeed, Hizukuri (1985) found that the chain length of amylopectin is a basic factor in the determination of the crystalline type of the starch.

On the basis of the double helix concept (Kainuma and French, 1972), several molecular models have been proposed for the unit cell structures that would satisfy the X-ray and electron diffraction experimental data. As proposed by Imberty *et al.* (1987, 1988), the double helices in both A and B types would be identical, but the mode of packing of the helices and the water content would differ (Fig. 4b). The A and B patterns represent true crystalline forms of starch, but the C form is a composite, containing elements of A and B. Many different structures have been proposed to explain the crystalline patterns (Banks and Muir, 1980; French, 1984), but it seems that the patterns are a result of a combination of factors, including the chain length of the amylopectin, helix packing, and water of crystallization (Hizukuri, 1986). The A pattern is more frequent in cereal starches, whereas the B pattern is found in potato and amylomaize starch. The C pattern can be obtained by mixing maize and potato starches (Hizukuri *et al.*, 1961), but it is also found in nature—for example, in smooth-seeded peas and in bean starches. Heat-moisture treatment can change the X-ray diffraction pattern from the B to the A pattern. Plants producing starch giving a B pattern can produce starch with an A pattern if they are grown at higher temperatures or if the isolated starch is partly dehydrated. The crystallinity of starch granules can be destroyed mechanically; for example, ball milling at room temperature will destroy both the birefringence and the X-ray pattern.

The orientation of the principal axis of the crystallites is radial with respect to the hilum (center) of the granule (French, 1972). Small-angle X-ray scattering data suggest the existence of a 9-nm repetitive unit that is found in all plants, implying the presence of a highly ordered biosynthetic pathway that is well conserved throughout the plant kingdom (Jenkins *et al.*, 1993). This repetitive unit is composed of an amorphous and a crystalline lamella. Although the sum of both lamellae remains constant (9 nm), the relative size of each in the repetitive unit is under genetic control. Lengths of 4 to 6 nm have been reported for the size of the crystalline lamella, and this would amount to a linear α-1,4-glucan of a size ranging from 12 to 18 glucose residues. Powder diffraction patterns of native starch have been used to determine the three-dimensional structures of the crystalline lamella

(reviewed by French, 1984; Imberty *et al.*, 1991; Hizukuri, 1995), and three types of diffraction patterns (A, B, C) were obtained. Each of these patterns is interpreted as the packing of linear (unbranched) parallel glucan double helices.

Amylopectin molecules are very large, flattened disks consisting of α-(1,4)-glucan chains joined by frequent α-(1,6)-branch points (Fig. 3). The chain that contains the single reducing end group is called the C-chain, to which all the other chains are ultimately attached (Fig. 5). The A-chains carry no branch points and are attached to B-chains, which have one or more branch points and are themselves attached to other B-chains or to the one C-chain (Peat *et al.*, 1952). Many models of amylopectin structure have been proposed (Fig. 5a), but of these the most satisfactory models, those that fit the experimental data available, are those proposed by Robin *et al.* (1974), Manners and Matheson (1981), and Hizukuri (1986; Fig. 5b). The arrowheads indicate the presence of a branching point [i.e., an α-(1,6) bond], and the branched regions of amylopectin are amorphous. The potentially crystalline clusters of A- and B-chains—the short, linear chains beyond the branch points that can form left-handed, parallel-stranded double helices—are also shown. The size of the crystallites is derived from the average chain length determined experimentally, and the ratio of A- to B-chains in the model can also be measured by enzymatic hydrolysis. Highly purified forms of the debranching enzymes isoamylase and pullulanase, and the chain-shortening β-amylase, each with well-defined specificities, are used to elucidate structural features of amylose, amylopectin, and the intermediate fraction. The products of these treatments are then identified by chromatography (Fig. 6; Table III). Hizukuri (1986) observed that size-exclusion chromatography of the products of isoamylase action on amylopectin had a polymodal distribution (Fig. 6a); there are essentially five peaks (A, B1, B2, B3, and B4) with chain lengths as indicated. The model proposed by Hizukuri (Fig. 5b) takes into account this information, as the polymodal distribution in the chromatogram supports his idea of a cluster structure: 80 to 90% of the chains (A + B1) span a single cluster, about 10% (B2) would span (and connect) two clusters, 1 to 3% would span three clusters, and only 0.1 to 0.6% would connect four or more clusters. High-performance anion chromatograhy (HPAC) is another methodology that has proven to be a useful and sensitive tool for studying the structure of the linear chains released by debranching amylopectin and related carbohydrates (Fig. 6b).

The adjacent branch structures in amylopectin would form double helices that are organized in a crystalline structure (see preceding), provided that the various chains are of suitable length.

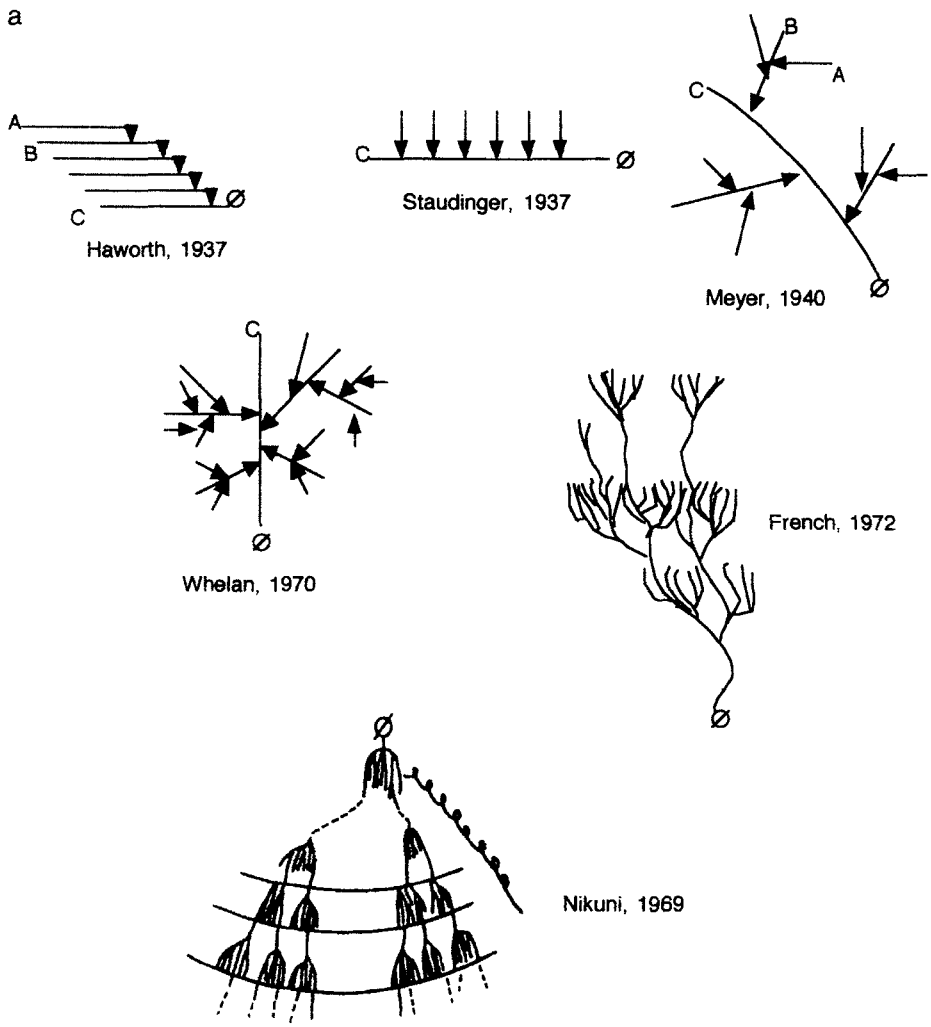

FIG. 5. (a) Historical evolution of the models for the structure of amylopectin as proposed by several workers; what varies in each model is the arrangement of the linear α-(1,4)-glucan chains and how they are joined by α-(1,6)-glycosidic linkages (arrowheads). (b) The model of Hizukuri (1986) showing A-, B_1-, B_2-, and B_3-chains (the very long B_4-chains are not illustrated) is the one more broadly accepted. "A" indicates A-chains whereas "B1", "B2", and "B3" are the B-chains; the C-chain has the only reducing end group, Ø, in the polysaccharide. The B3-chains are longer than the B2-chains, which are longer than the B1-chains. The B2-, B3-, and B4-chains extend into 2, 3, and 4 cluster regions, respectively. The average chain lengths are 19 for B1, 41 for B2, 69 for B3, and 104 for B4. The shortest chain length is for the A-chains, which have no branch points.

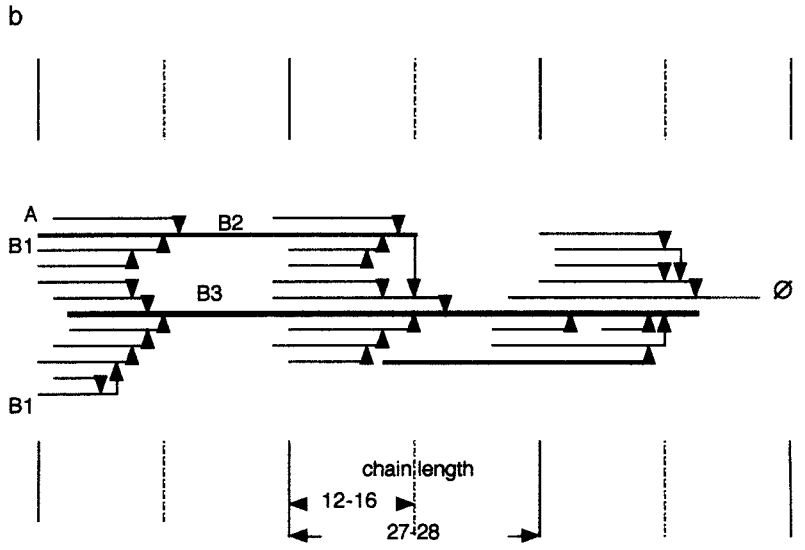

FIG. 5. (*Continued*).

The linear chains in the amylopectin form red to purple polyiodide complexes (Krisman, 1972) with a λ_{max} of between 530 and 585 nm. Altogether, the iodine binding capacity of amylopectin is very low, varying between 0 and 2.5 g/g depending on the botanical source of the amylopectin (Table IV). There are different kinds of atypical (anomalous) amylopectins (Baba *et al.*, 1987; Hizukuri, 1986; Takeda and Hizukuri, 1987), but they all bind more iodine and give a higher λ_{max} with I_2/KI solutions, leading to errors in determining the amylose content in starch when using the blue value (BV) or iodine affinity (IA) in the calculations. The IA is measured by amperometric titration; as iodine is added, the electric current does not increase until all the amylose molecules are saturated with iodine. Conversely, amylopectin cannot easily form the helical complex because the short chains and many branch linkages interfere with its formation. The BV is the absorbance at 680 nm of the iodine–glucan complex, under defined conditions, and can also be used to calculate the approximate proportion of amylose and amylopectin. One of the factors that affects the reliability of the IA and the BV as indicators of the proportion of amylose in the starch is the presence of lipids (relatively high in cereals), which also bind iodine.

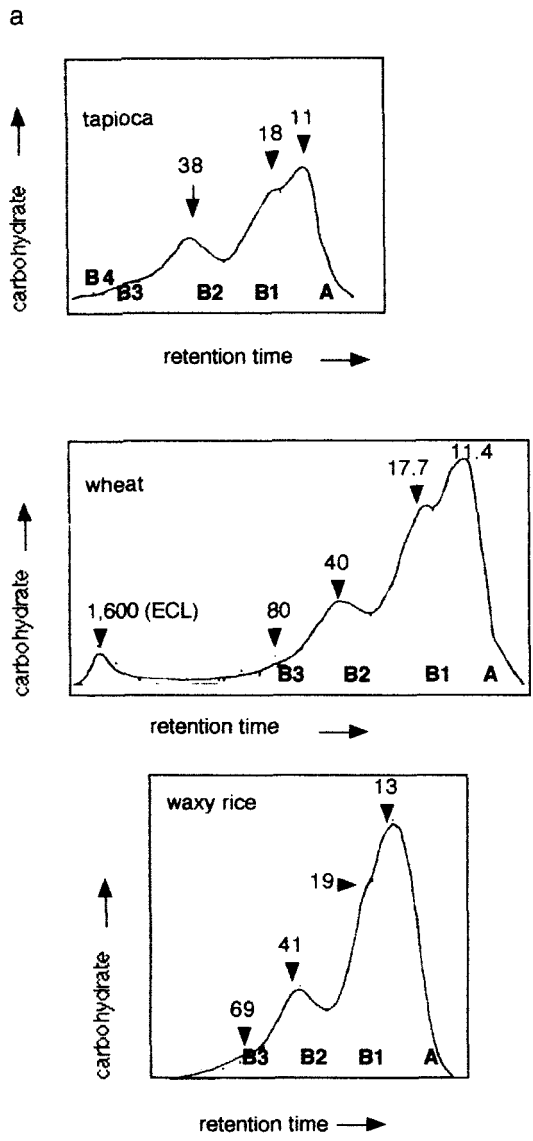

FIG. 6. (a) Size-exclusion high-performance liquid chromatography of amylopectins after debranching by isoamylase, showing the different chain length distributions for amylopectin from different species. The lower the retention time, the longer the debranched side chain. After Hizukuri (1995). (b) High-performance ion-exchange chromatography (using pulsed amperometric detection) of the linear chains obtained by debranching of amylopectin using isoamylase. The numbers indicate the degree of polymerization of the linear chains, and the height of the peak the relative amount of each chain length within the amylopectin (i.e., chain length distribution). The lower the retention time, the shorter the side chain. After Koizumi *et al.* (1991).

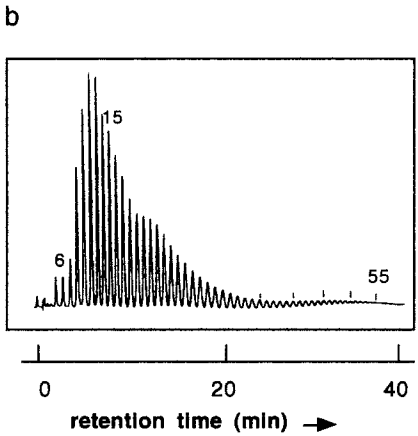

FIG. 6. (*Continued*).

III. MOLECULAR ORIENTATION IN THE GRANULE

Several levels of structural organization exist within the starch granule, as shown by the use of different methodologies. For example, starch granules show birefringence patterns in plane-polarized light that resemble maltese crosses (Fig. 1). Birefringence indicates a great degree of order in the molecular orientation, a characteristic that is independent of crystallinity; that is, noncrystalline polymers can show birefringence if their long axes are oriented by applied stress. The analysis of starch birefringence indicates that the chain axis of the polysaccharide is radially arranged. The

TABLE III

GENERAL PROPERTIES OF AMYLOPECTINS FROM DIFFERENT SOURCES[a]

Botanical source	B value	Iodine affinity	λ_{max}	β-Amylolysis limit (%)	Chain length	$[\eta]$	P0 (ppm)	P6 (ppm)
Wheat	0.098	0.89	552	57	20	145	9	<0.1
Maize (wild type)	0.11	1.10	554	59	22	—	14	4
Amylomaize	0.427	3.60	573	61	30	—	110	54
Rice	0.049	0.39	535	59	20	137	11	11
Barley	0.090	0.73	540	60	20	147	—	—
Sweet potato	0.166	0.44	—	56	22	180	135	101
Tapioca	0.104	—	—	57	21	—	—	—
Potato	0.245	0.06	—	56	23	—	900	840

[a] Data from Hizukuri (1995).

TABLE IV

DISTRIBUTION OF CHAIN LENGTHS OF AMYLOPECTINS FROM DIFFERENT SOURCES[a]

Fraction	Whole	A	B1	B2	B3	B4	Long chain	A/B
Waxy rice								
Chain length (max)		13	19	41	69			
Weight (%)	100	50	26.2	18.9	4.1	0.8		
Mole (%)	100	69.2	21.7	8.0	1.0	0.1		2.2
Wheat								
Chain length (max)		11	18	40	80			
Weight (%)	100	42	32.7	16.7	3.2	0.9	4.5	
Mole (%)	100	63.2	28.4	7.5	0.8	0.1	0	1.7
Tapioca								
		11	18	38	62			
	100	38.5	32.5	23.0	5.1	0.9		
	100	59.6	28.7	10.2	1.4	0.1		1.5
Potato								
		16	19	45	74			
	100	27.8	34.9	26.0	9.1	2.3		
	100	44.2	38.1	14.0	3.1	0.6		0.79

[a] Data from Hizukuri (1995).

constraints imposed on the growth of the starch granule by the shape of the plastid in which it is formed determine its shape, but do not seem to affect the internal arrangement of the molecules, which is analogous to that shown by semicrystalline polymers growing in solution.

Optical microscopy, scanning electron microscopy, or transmission electron micrographs of etched and stained thin sections of starch granules show a layered "growth ring" structure with ring spacings on the order of 1 μm. These rings are particularly visible after chemical treatment, and they represent shells of higher and lower starch content produced by the rate or mode of starch deposition. According to French (1984), growth rings would represent periodic growth and, with the cereal starches, daily fluctuations in the amount of carbohydrate available for starch deposition. The arms of the polarization cross are always perpendicular to the growth rings, indicating that the optic axes of the starch crystallites are aligned perpendicularly to the growth rings and the granule surface.

It seems that the molecules of amylose and amylopectin are arranged radially within the granule, at a right angle with the surface, and with their hypothetical single reducing end group toward the hilum or center of the granule (see Chapter 7, Initiation of Starch Synthesis). Growth of the granule is by aposition at the outer nonreducing end.

Scattering and diffraction measurements coupled with electron microscopy have shown layering within the granule, with unit blocks spaced at regular intervals of about 10 nm. These would be the result of regular spacing between clusters of partially crystalline amylopectin branches (Manners, 1989; Oostergetel and van Bruggen, 1989).

The model proposed by Kainuma and French (1972) attempts to explain the structure of the granule in relation to the polysaccharides that constitute it. The model shows the direction of growth of the amylopectin molecule; the crystal would grow at a right angle to the length of the molecule. Striations in the granule are caused by alternations of crystalline and amorphous zones, and these striations can be better observed with scanning electron microscopy of sections of granules partially digested with amylases or by acid treatment, which preferentially attacks the amorphous regions. At even higher magnification, and using transmission electron microscopy, it is possible to distinguish heterogeneities that are of the same magnitude as the A- and B-chain segments of amylopectin that are capable of forming crystalline parallel-stranded double helices. Order of the crystallites is probably responsible for the birefringence mentioned previously.

IV. METHODOLOGY AND NOMENCLATURE USED IN STARCH ANALYSIS

Much of what we know about starch structure is the result of the painstaking research of the pioneers of polysaccharide chemistry in the 1930s and 1940s: W. N. Haworth, E. L. Hirst, D. J. Bell, E. G. V. Percival, and others, who by using periodate oxidation, methylation, and paper chromatography established the basis for others, such as D. French, W. J. Whelan, and D. J. Manners to build on in the 1950s and 1960s. The purification and characterization of amylolytic enzymes from several sources have made possible the use of enzymatic, rather than chemical, identification of the starch components. Why is a detailed identification of the starch components so important? When a mutation affecting a particular enzyme results in changes in the seed appearance, the resulting changes in starch structure may be subtle: for example, a slight decrease in the average chain length of amylopectin or a small increase in the proportion of amylose to amylopectin. The resulting changes in the pasting quality of the starch, and/or its temperature of gelatinization, may be important to the industrial users. Identification of the enzymatic changes and of the consequent modification of the starch formed will help to illuminate the process of starch biosynthesis and to facilitate the task of "designing" a raw material that matches the needs of the industrial user.

V. OTHER CONSTITUENTS OF THE STARCH GRANULE

Although the proportion of amylose and amylopectin and their properties are paramount in determining the characteristics of the starch, minor constituents of the starch granule seem to affect the properties relevant to its use as food and in industrial applications. These minor constituents are not just contaminants (e.g., particles of bran that remained after scraping the wheat grain from the outer bran layer), but materials that are associated with the surface of the grain or are true internal components. The surface components may be remainders of the amyloplast in which the starch grain was formed (during the grain maturation, the amyloplast envelopes are disrupted and may remain on the surface of the granule), or the components may be endosperm proteins that became strongly attached to the granule during the maturation and drying of the grain. The surface components can be washed with water or salt solutions. Conversely, the internal components are part of the granule, and to extract them the starch granule must be disrupted.

VI. LIPIDS

Cereal starches contain low levels of lipids (0.5–1%), which are generally polar lipids requiring polar solvents such as methanol–water for extraction. Lipid content increases with amylose content, and unless the granule integrity is disrupted, the lipids remain inaccessible to normal fat solvents, suggesting that they are present as an amylose inclusion complex. Noncereal starches contain essentially no lipids.

Starches contain phosphorus, nitrogen, and very low amounts of other minerals. In the cereals, most of the phosphorus is in the form of phospholipids, whereas in potato starch the phosphorus is esterified to certain glucose residues in the polysaccharide.

VII. PHOSPHORUS

Generally, the phosphorus content in starches is associated with different pasting properties, and it confers a larger ion binding capacity. In wheat and corn starch, phosphorus is present largely or wholly as adsorbed phosphatides (extractable with boiling 85% methanol) associated preferentially with the amylose fraction.

Many amylopectins, but not amyloses, contain small amounts of esterified phosphate groups, present as residues of glucose 6-phosphate. Adsorbed

phosphatides can be removed with suitable solvents, but esterified phosphate, such as that present in potato amylopectin, remains. The content of esterified phosphate varies between 200 and 1000 ppm in potato amylopectin and 40 and 150 ppm in starch from other tubers and roots but is very low in cereal starch (less than 20 ppm), with the exception of amylomaize, which contains 110–260 ppm (Takeda *et al.*, 1993).

Esterified phosphates have a marked effect on the physical properties of amylopectin and the extent of degradation by α- and β-amylase. After amylolysis, phosphorus is concentrated into the limit dextrins. The origin of the esterified phosphate, which would be close to the branching points, is not known. In general, glucose 6-phosphate and other glycolytic intermediates are not substrates for starch-synthesizing enzymes, and it still remains to be determined whether the glucose 6-phosphate residues are incorporated into a growing (1→4)—α-D-glucan chain or arise from enzymatic phosphorylation of certain residues in amylopectin. Whatever the mechanism, it is not random, since one-third of the phosphate groups are present in the inner regions of B-chains and two-thirds are present in the outer parts of the B-chains and in the A-chains.

The position of the esterified phosphates at C-6 can be determined using acid hydrolysis followed by enzymatic methods, but ^{31}P NMR has been used to determine the position of ester linkages at other positions, such as C-3 and C-2 (Lim and Seib, 1993; Kasemsuwan and Jane, 1994).

Phosphorus content of potato starch (Geddes *et al.*, 1965) varies during development of the tubers; it increases from being undetectable in the amylose of tubers of 1-cm diameter, to 0.005% in tubers 8 to 9 cm in diameter, and, in amylopectin, from 0.029% in the 1-cm diameter to 0.049% in the largest (8- to 9-cm tubers).

Total phosphorus is determined as inorganic phosphate after treatment with hot perchloric acid. Phosphorus in D-glucose-6-phosphate residues is assayed using D-glucose-6-phosphate dehydrogenase.

VIII. PROTEINS

It has been known for many years that even after exhaustive washes, starch still contains small amounts of noncarbohydrate elements. Lipids account for most of the phosphorus and about one-third of the nitrogen present in wheat starch (Table I). However, amino acids have been recovered from hydrolyzed starch, indicating that the balance of nitrogen is present as proteins. In the case of well-isolated, nondamaged wheat starch, proteins represent 0.15 to 0.2% of its weight. This is a very small proportion of the flour protein, which comprises all the proteins contained in the

endosperm, including storage proteins. However, small, granule-bound proteins have attracted attention for several reasons. Some, if not all, of the starch proteins are likely to be implicated in the formation of the granule. The drastic methodology required to extract these proteins from the granule makes their purification and characterization a difficult task. Some information is available on the waxy protein (see the chapter, "Starch Synthases") and on the pollen starch protein implicated in human allergy.

Proteins affect the milling and baking properties of the starch (Gough *et al.*, 1985; Greenwell *et al.*, 1985); the presence of one polypeptide in particular, of Molecular Weight 15,000, seems to determine the degree of "hardness" of wheat endosperm.

Inhalation of pollen present in the air provokes IgE-mediated responses of hay fever and allergic asthma in about 20% of humans. The allergens present in the pollen of rye grass (*Lolium perenne,* one of the grasses implicated in this response and the one that produces the greatest amount of pollen) are a group of low-molecular-weight proteins (Singh *et al.*, 1990). Electron microscopy shows that one of them, *Lolp*Ib, of Molecular Weight 31,000 and isoelectric point 9.0, is associated with the starch granules. *Lolp*Ib accumulates as the pollen grain matures, but its physiologic role is unknown. On contact with water the pollen grains burst, releasing the starch granules (about 1000 per grain), which are small enough to pass the barriers present in the mucose membranes, and amplifying the allergic response (Singh *et al.*, 1991).

FURTHER READINGS

These sources provide additional in-depth coverage of this topic. For complete reference, please see the Reference section at the end of the book.

Banks, W., and Greenwood, C. T. (1975)
Banks, W., and Muir, D. D. (1980)
Fitt, L. E., and Snyder, E. M. (1984)
French, D. (1984)
Hizukuri, S. (1995)
Imberty, A., Buléon, A., Tran, V., and Perez, S. (1991)
Kainuma, K. (1988)
Manners, D. J. (1985)
Morrison, W. R., and Karkalas, J. (1990)
Whistler, R. L. (1964)